

CLAIMS:

1. A polysaccharide sponge characterized by having: (i) an average pore size in the range between about 10  $\mu\text{m}$  to about 300  $\mu\text{m}$ ; (ii) an average distance between the pores being the wall thickness of the pores in the range between about 5  $\mu\text{m}$  to about 270  $\mu\text{m}$ ; and (iii) an E-modulus of elasticity being a measure of the rigidity of the sponge in the range of about 50 kPa to about 500 kPa.
2. A polysaccharide sponge according to claim 1, wherein said sponge comprises a polysaccharide selected from the group comprising the polyanionic polysaccharides: alginates, gellan, gellan gum, xanthan, chitosan, agar, carrageenan and the polycationic polysaccharide: chitosan.
3. A polysaccharide sponge according to claim 1 or claim 2, wherein said sponge comprises an alginate selected from the group of alginates characterised by having: (i) a mannuronic acid (M) residue content in the range of between about 25% and about 65% of total residues; (ii) a guluronic acid (G) residue content in the range of between about 35% and about 75% of total residues; (iii) a M/G ratio of about 1/3 and about 1.86/1; and (iv) a viscosity of the final alginate solution having 1% w/v alginate, from which the sponge is obtained in the range between about 50 cP to about 800cP.
4. A polysaccharide sponge according to claim 3, wherein said sponge comprises an alginate derived from brown sea algae selected from the group consisting of alginate Protanal<sup>TM</sup> LF 120 (LF 120) derived from Laminaria hyperborea, alginate Protanal<sup>TM</sup> LF 20/60 (LF 20/60) derived from Laminaria hyperborea, alginate MVG<sup>TM</sup> (MVG) derived from Laminaria hyperborea, alginate Pronatal<sup>TM</sup> HF 120 (HF 120) derived from Laminaria hyperborea, alginate Pronatal<sup>TM</sup> SF 120 (SF 120) derived from Laminaria hyperborea, alginate Pronatal<sup>TM</sup> SF 120 RB (SF 120 RB) derived from Laminaria hyperborea, alginate Pronatal<sup>TM</sup> LF 200 RB (LF 200 RB) derived from Laminaria hyperborea, alginate Manugel<sup>TM</sup> DMB (DMB) derived from Laminaria hyperborea, Keltone<sup>TM</sup> HVCR (HVCR)

derived from Macrocystis pyrifera, and Keltone™ LV (LV) derived from Macrocystis pyrifera.

5. A polysaccharide sponge according to claim 4, wherein said sponge comprises an alginate selected from the group consisting of said LF 120, LF 20/60 and HVCR.

6. A polysaccharide sponge according to any one of claims 3 to 5, wherein said alginate is used in the form of a sodium alginate solution having a concentration of alginate between about 1% to about 3% w/v to provide an alginate concentration between about 0.1% to about 2% w/v in the final solution from which the sponge is obtained.

7. A polysaccharide sponge according to any one of claims 1 to 5, wherein said sponge further comprises a cross-linking agent selected from the group consisting of the salts of calcium, copper, aluminum, magnesium, strontium, barium, tin, zinc, chromium, organic cations, poly(amino acids), poly(ethyleneimine), poly(vinylamine), poly(allylamine), and polysaccharides.

8. A polysaccharide sponge according to claim 7, wherein said sponge further comprises a cross-linking agent selected from the group consisting of calcium chloride ( $\text{CaCl}_2$ ), strontium chloride ( $\text{SrCl}_2$ ) and calcium gluconate (Ca-Gl).

9. A polysaccharide sponge according to claim 7 or claim 8, wherein said cross-linker is used in the form of a cross-linker solution having a concentration of cross-linker sufficient to provide a cross-linker concentration between about 0.1% to about 0.3% w/v in the final solution from which the sponge is obtained.

10. A polysaccharide sponge according to any one of claims 1 to 9, wherein said sponge is prepared from a polysaccharide solution with or without the addition of a cross-linker.

11. A polysaccharide sponge according to claim 10, wherein said sponge is an alginate sponge prepared from an alginate solution with or without the addition of a cross-linker and wherein said final alginate solution with or without cross-linker from which said sponge is obtained is selected from the group of final solutions, having concentrations of alginate or alginate and cross-linker, consisting of: (i) LF 120 alginate 1% w/v without cross-linker; (ii) LF 120 alginate 1% w/v and Ca-Gl 0.1% w/v; (iii) LF 120 alginate 1% w/v and Ca-Gl 0.2% w/v; (iv) LF 120 alginate 1% w/v and  $\text{SrCl}_2$  0.15% w/v; (v) LF 120 alginate 1% w/v and  $\text{CaCl}_2$  0.1% w/v; (vi) LF 120 alginate 0.5% w/v and Ca-Gl 0.2% w/v; (vii) LF 20/60 alginate 1% w/v and Ca-Gl 0.2% w/v; (viii) HVCR alginate 0.5% w/v and Ca-Gl 0.2% w/v; and (ix) HVCR alginate 1% w/v and Ca-Gl 0.2% w/v.

12. A polysaccharide sponge according to claim 11, wherein said sponge is obtained from a final solution of LF 120 alginate 1% w/v and Ca-Gl cross-linker 0.2% w/v.

13. A polysaccharide sponge according to claim 11, wherein said sponge is obtained from a final solution of HVCR alginate 1% w/v and Ca-Gl cross-linker 0.2% w/v.

14. A polysaccharide sponge according to any one of claims 1 to 13 for use as a matrix, substrate or scaffold for growing mammalian cells *in vitro*.

15. A polysaccharide sponge according to any one of claims 1 to 13 for use as a matrix, substrate or scaffold for implantation into a patient to replace or repair tissue that has been removed or damaged, wherein said implanted sponge is a substrate, matrix or scaffold for surrounding tissue to invade it, proliferate thereon and replace the damaged or removed tissue, or wherein said implant is an initial substrate for vascularization by the surrounding host tissue and the vascularized implant then serves as a substrate to receive injected cells of choice from the host, or grown *in vitro*, said injected cells being capable of rapid acclimitization and proliferation on the vascularized sponge to rapidly replace the damaged or removed tissue.

16. A polysaccharide sponge according to any one of claims 1 to 13 for use as an implanted support for therapeutic drug delivery into a desired tissue, said drug delivery being by way of the action of genetically engineered cells or natural cells carried by said sponge and expressing said therapeutic drugs, said cells expressing said drug or expressing regulatory proteins to direct the production of the drug endogenously in said tissue.

17. A polysaccharide sponge according to claim 16, wherein said therapeutic drug expressed by said cells carried in said sponge is a therapeutic protein, wherein said cells express said protein or express regulatory proteins to direct the production of said protein endogenously in the tissue into which said sponge is implanted.

18. A polysaccharide sponge according to any one of claims 1 to 13 for use as a matrix, substrate or scaffold for in vitro culturing of plant cells and algae.

19. A polysaccharide sponge according to any one of claims 1 to 13 for use as a matrix, substrate or scaffold for the delivery to a tissue or organ of genetically engineered viral vectors, non-viral vectors, polymeric microspheres and liposomes all encoding or containing a therapeutic agent for said tissue or organ.

20. A polysaccharide sponge according to any one of claims 1 to 13 for use as a matrix, substrate or scaffold for in vitro fertilization of mammalian oocytes.

21. A polysaccharide sponge according to any one of claims 1 to 13 for use as a matrix, substrate or scaffold for storage of fertilized mammalian oocytes or other mammalian cells cultured in vitro.

22. A polysaccharide sponge according to any one of claims 1 to 13 for use as a matrix, substrate or scaffold for the transplantation of cells grown on or within said sponge in vitro into a tissue of a patient in need of said cells as a result of tissue damage, removal, or dysfunction.

23. A process for producing a polysaccharide sponge according to any one of claims 1-22, comprising:

- (a) providing a polysaccharide solution containing about 1% to about 3% w/v polysaccharide in water;
- (b) diluting said polysaccharide solution with additional water when desired to obtain a final solution having about 0.5% to about 2% w/v polysaccharide, and subjecting said solution of (a) to gelation, to obtain a polysaccharide gel;
- (c) freezing the gel of (b); and
- (d) drying the frozen gel of (c) to obtain a polysaccharide sponge.

24. A process according to claim 23, further comprising the addition of a cross-linker to said polysaccharide solution of (a) during the step of gelation (b), said cross-linker being added in an amount to provide a concentration of cross-linker in the final solution being subjected to gelation of between about 0.1% to about 0.3% w/v.

25. A process according to claim 23 or 24, wherein said polysaccharide solution of (a) is prepared by dissolving the polysaccharide in powdered form in double distilled water in amounts to yield a concentration between about 1% to about 3% w/v polysaccharide in said solution, said polysaccharide solution being mixed in a homogenizer at about 25000 RPM for about 30 minutes at room temperature.

26. A process according to any one of claims 23-25, wherein the gelation step (b) is by intensive stirring of the polysaccharide solution in a homogenizer at about 31800 RPM for about 3 minutes, and wherein when a cross-linker is added to the solution, said cross-linker is added very slowly during said intensive stirring of the polysaccharide solution.

27. A process according to any one of claims 23 to 26, wherein said polysaccharide is an alginate of claim 4.

28. A process according to claim 27, wherein in said process, the final solutions subjected to gelation in step (b) are selected from the group consisting of: (i) LF 120 alginate 1% w/v without cross-linker; (ii) LF 120 alginate 1% w/v and Ca-Gl 0.1% w/v; (iii) LF 120 alginate 1% w/v and Ca-Gl 0.2% w/v; (iv) LF 120 alginate 1% w/v and  $\text{SrCl}_2$  0.15% w/v; (v) LF 120 alginate 1% w/v and  $\text{CaCl}_2$  0.1% w/v; (vi) LF 120 alginate 0.5% w/v and Ca-Gl 0.2% w/v; (vii) LF 20/60 alginate 1% w/v and Ca-Gl 0.2% w/v; (viii) HVCR alginate 0.5% w/v and Ca-Gl 0.2% w/v; and (ix) HVCR alginate 1% w/v and Ca-Gl 0.2% w/v.

29. A process according to any one of claims 23-28 wherein said freezing step (c) is by rapid freezing in a liquid nitrogen bath at about  $-80^\circ\text{C}$  for about 15 minutes.

30. A process according to any one of claims 23-28 wherein said freezing step (c) is by slow freezing in a freezer at about  $-18^\circ\text{C}$  for about 8-24 hours.

31. A process according to any one of claims 23-30 wherein said drying step (d) is by lyophilization under conditions of about 0.007 mmHg pressure and at about  $-60^\circ\text{C}$ .

32. A process according to any one of claims 23-31 wherein the final polysaccharide solution with or without cross-linker is poured into a vessel of desired shape before commencement of the intensive stirring of the gelation step (b). said vessel having a shape that is desired for the shape of the polysaccharide sponge.

33. Use of a polysaccharide sponge according to any one of claims 1-13 as a matrix, substrate or scaffold for the *in vitro* growth of mammalian cells, plant cells, algae, or for the *in vitro* fertilization of mammalian oocytes.

34. Use of a polysaccharide sponge according to claim 33 for the *in vitro* growth of fibroblast cells.

35. Use of a polysaccharide sponge according to claim 33 for the *in vitro* growth of hepatocytes.

36. Use of a polysaccharide sponge according to any one of claims 1 to 13 as a matrix, substrate or scaffold for implantation into a patient according to any one of claims 15-17.

37. Use of a polysaccharide sponge according to any one of claims 1 to 13 as a matrix, substrate or scaffold for the transplantation of cells grown on said sponge in vitro into a tissue of a patient in need of said cells as a result of tissue damage, removal, or dysfunction.

38. Artificial skin comprising a polysaccharide sponge according to any one of claims 1 to 13 and dermal fibroblast cells grown on said sponge in vitro to the stage wherein said cells are in an active proliferating stage suitable for transplantation to a patient in need of said artificial skin.

39. An artificial organ equivalent comprising a polysaccharide sponge according to any one of claims 1 to 13 and representative cells of said organ, said cells having been grown on said sponge in vitro to the stage wherein said cells are fully active and equivalent to the active cells of said organ, said artificial organ being suitable for transplantation or implantation into a patient in need thereof following organ damage, removal or dysfunction.

40. An artificial organ equivalent according to claim 39 being an artificial liver equivalent, wherein said cells grown on said sponge are hepatocytes at a stage in which said hepatocytes are active and function in an equivalent manner to hepatocytes in vivo and are suitable for transplantation or implantation into a patient suffering from liver dysfunction, damage or at least partial removal.